July 11, 1972

Dr. Ernest Winocour
Dr. Maxine Singer
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Dear Ernest and Maxine:

Thank you for your recent letter. Your results are very interesting to me and are similar, but not identical, to what we have observed on passing our cloned stock of SV40 at high multiplicity. We have passed the virus serially some fifteen times and have followed the changes in digest pattern of the Hirt-extracted SV40 DNA at each passage. There is a striking change consisting of progressive loss of almost all the initial fragments and the sequential appearance of specific new fragments. By the 12th or 13th passage there are five main bands by electrophoresis, only one of which corresponds to an initial fragment. We are just now ready to hybridize these new fragments with original fragments and with cellular DNA. Our results indicate that there are preferred sites of deletions (and/or substitutions) in the early passage DNA, since particular groups of fragments disappear first and specific new ones appear. It will be interesting to compare our final results with yours, since there are some differences already apparent. It should be possible for each of us to map the preferential deletion and substitution sites in our particular virus stocks.

I am sorry the enzyme preparation has not been satisfactory. We will be making another preparation later this summer and should be able to tide you over.

In answer to your question about strain differences, we have examined to date the following: small plaque (776, from K. Takemoto); large plaque (derived from small plaque by Takemoto); minute plaque (also from small plaque by Takemoto); SV68 (a rescued virus); five other rescued strains; a human isolate of an SV40-like virus from a patient with progressive multifocal leukoencephalopathy; another small plaque strain (776, grown by Malcolm Martin from Takemoto's seed). In summary, there are frequent differences, mostly limited to

fragments C and F, but rarely involving others. I believe these are "variable" regions probably altered by integration-excision as Ernest suggested some time ago, or by viral DNA recombination. Relevant to this last possibility is your question about homology between fragments. We have not systematically hybridized one fragment with another, but have melted and reannealed a digest without finding any new fragments. We should do it systematically, though I suspect the sequencers will soon answer this question. (Sherman Weisman and Walter Fiers are analysing RNA transcripts of the fragments.)

I look forward to seeing Maxine on your return, and also hope that Ernest will spend some time with us when you next come to the United States.

Sincerely,

Daniel Nathans

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